Reference: *Biol. Bull.*, 153: 560–576. (December, 1977)

GENETIC VARIATION WITHIN AND BETWEEN GEOGRAPHICALLY SEPARATED POPULATIONS OF THE SEA URCHIN, *ARBACIA PUNCTULATA*

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The sea urchin, *Arbacia punctulata*, is one of the most widely studied organisms in developmental and cellular biology. Knowledge of its development from the fertilized egg through the pluteus stage is considerable and continually increasing. What is understood of the genetics and population biology of this species is, however, limited and dates back primarily to the early works of Jackson (1912, 1927), Mortensen (1935), and Harvey (1956). The species is a benthic marine invertebrate, dioecious and with an enormous reproductive potential. Harvey (1956) reports that a single female contains eight million eggs during a reproductive season. Fertilization takes place in the water column, where the fertilized egg undergoes a series of cleavages, forming a blastula. Gastrulation follows and within 24–48 hours a free-swimming pluteus larva is formed which feeds on the plankton. Metamorphosis into the adult form may occur after 3–8 weeks (Cameron and Hinegardner, 1974; Harvey, 1956; Marcus, 1976). The duration of the larval phase is dependent on various environmental factors such as food and temperature (Hinegardner, 1969, 1975).

As is characteristic of many marine organisms, *A. punctulata* has a latitudinally broad range. Its recorded geographic distribution is from Woods Hole, Massachusetts, U. S. A. to Venezuela, South America, including Trinidad, Tobago, Curacao, and the Yucatan (see Harvey, 1956). The distribution of the species is not continuous within this range, however, which may be due to several factors such as low salinity and unsuitable substrates (see Marcus, 1976; Hedgepeth, 1953). Throughout this extensive range, the genus *Arbacia* is represented by a single polytypic species, *punctulata* (Mortensen, 1935). The possible existence of physiological races, ecotypes, or subspecies has not previously been examined for this species. The question that arises is whether or not any exchange between the various populations is occurring, and, if so, what effect it has on maintaining genetic continuity within the species. Exchange could be achieved by dispersal of the planktonic larval stage and, within a much more limited area, by adult migration. Morphological variation between geographically distant populations has been described (Jackson, 1912, 1927; Clark, 1923). These morphological differences may reflect basic genetic differences between the populations. On the other hand, because environmental conditions are not necessarily similar, the variation may be environmentally induced.

The present study was initiated to characterize the genetic composition of geographically separated populations of *A. punctulata*, as shown by protein variation, and to relate any observed patterns of genetic variation to environmental factors and possible routes of larval transport. It is necessary to discriminate between genet-

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ically and environmentally determined phenotypic traits before an understanding of evolutionary divergence within a species is possible.

Materials and Methods

Genetic variation, inferred from protein variation was determined in four geographically separated populations of *Arbacia punctulata* from: Woods Hole, Massachusetts (collected in January, 1976, by the Marine Biological Laboratory Supply Department); Wall, New Jersey (collected in December, 1975, by Connecticut Valley Supply); Willis Wharf, Virginia (collected in December, 1975, by the H. M. Terry Oyster Company); and Beaufort, North Carolina (collected in November, 1975, by N. Marcus). Upon arrival at Yale University, New Haven, Connecticut, the urchins were transferred into recirculating salt water aquaria within a constant temperature chamber at 19° C and allowed to acclimate for a minimum period of two weeks prior to electrophoresis.

At the time this study was conducted (October, 1975 to August, 1976), *A. punctulata* was not available from the Gulf of Mexico despite numerous attempts to secure them from much of the Gulf coast between Tampa, Florida and Port Aransas, Texas. The absence of the animal was attributed to a series of hurricanes occurring in the region during September and October, 1975. Repopulation of the area has been observed since November, 1976, and a small sample of urchins from the northern Gulf coast near Panama City, Florida (collected in December, 1976, by the Gulf Specimen Supply Company) has been studied. Protein variation was determined for PGM, HK, EST, and ACPH. The batch of urchins, however, were not healthy (dropping spines, absence of righting responses) and did not acclimate for a full two weeks.

Sample preparation

Each tissue sample was prepared from a freshly-killed sea urchin. The Aristotle's lantern (feeding apparatus) was removed from a slit made in the peristome, and the digestive tract was lifted out. The gut tissue was washed in extraction buffer (0.1 M Tris, pH 7.0—1.0 mM EDTA—25.0 mM 2-Mercaptoethanol—50.0 μM $NADP^+$) (Levin, Howland, and Steiner, 1972). Interference from food pellets was reduced by starving the animals during the acclimation period prior to electrophoresis. The tissue was blotted, and a portion (65.0–85.0 mg) was transferred to a polystyrene microcentrifuge tube (capacity 0.4 ml) containing 0.1 ml extraction buffer. The tissue was homogenized with a motor driven Plexiglass rod, and then centrifuged at 39,100 × *g* for 15 minutes. A portion (2.5 μl) of the clear supernatant was added to each gel slot (see below). The sequence of preparative steps outlined above were carried out over ice or under refrigeration. At the time of sample preparation each urchin was sexed. The size distribution of urchins examined was similar for all populations.

Gel preparation

Separation of proteins in the tissue homogenates was achieved with a horizontal microacrylamide gel apparatus (Ogita, 1975). Gels (1.0 mm thick) were made one day prior to electrophoresis and refrigerated until needed. The gels were prepared

from the following stock solutions: 47.5 g acrylamide, 2.5 g bisacrylamide in 250.0 ml deionized water; gel buffer; 1.0 ml of TEMED in 100.0 ml deionized water; 240.0 mg ammonium persulfate in 100.0 ml deionized water; and deionized water. Gels containing 5.0% or 6.5% (in parenthesis) acrylamide were prepared by mixing the stocks in the following proportions: 5.0 (6.5) : 5.0 : 5.0 : 2.5 : 2.5 (1.0).

Gel and electrode buffers

Five buffer system combinations were used: 1) gel buffer—0.11 M Tris, 0.062 M boric acid, 0.008 M NaCl pH 8.65 (Ogita, Yale University, personal communication), electrode buffer—0.3 M borate pH 8.0 (Shaw and Prasad, 1970); 2) gel buffer—0.18 M Tris, 0.1 M boric acid, 0.013 M NaCl pH 9.1, electrode buffer—0.3 M borate pH 8.0 (Shaw and Prasad, 1970); 3) gel buffer—0.32 M Tris glycine pH 8.8, electrode buffer—0.1 M glycine pH 8.6; 4) gel buffer—0.2 M Tris glycine pH 8.8, electrode buffer—0.1 M glycine pH 8.6; and 5) gel buffer—0.25 M Tris HCl pH 9.0, electrode buffer—0.5 M Tris HCl pH 9.0 (modified Shaw and Prasad, 1970).

Electrophoresis and enzyme detection

A single gel containing eight samples was inverted over buffer trays in a refrigerator at 2° C and electrophoresed at a constant voltage. Bromphenol blue and bovine serum albumin were used as the tracking standard.

At the conclusion of electrophoresis, the gel was removed and tested for enzyme activity by substrate specific staining at 37° C. Subsequently the gel was washed in water, soaked for one hour in glycerine (5.0%), and preserved permanently between two sheets of cellophane (Ogita, 1975).

Each tissue sample was tested for twelve enzyme systems. The electrophoretic conditions for each enzyme system (gel percentage, buffer system, magnitude of electrophoresis, and migration distance of the standard) are indicated below with the appropriate assay. The following assays were modified from Ayala, Powell, Tracey, Mourao, and Perez-Salas (1972): alkaline phosphatase (ALK), 6.5%, buffer system one, 70 volts, 34.0 mm, stain—25.0 mg α-napthyl phosphate, 25.0 mg Fast Blue BB, 20.0 mg $MnCl_2$, 20.0 mg $MgCl_2$, 500 mg NaCl, in 25.0 ml 0.05 M Tris pH 8.5; acid phosphatase (ACPH), 6.5%, buffer system three, 40 volts, 30.0 mm, stain—25.0 mg α-napthyl acid phosphate, 25.0 mg Fast Blue BB, in 25.0 ml 0.125 M acetate buffer pH 5.0; malic enzyme (ME), 6.5%, buffer system three, 40 volts, 33.0 mm, stain—5.0 mg $NADP^+$, 5.0 mg NBT, 1.5 mg PMS, 30.0 mg L-malic acid, 10.0 mg $MgCl_2$, in 25.0 ml of 0.05 M Tris buffer pH 9.2; malate dehydrogenase (MDH), 6.5%, buffer system three, 40 volts, 33.0 mm, stain—10.0 mg NAD, 5.0 mg NBT, 1.5 mg PMS, 30.0 mg L-malic acid, in 25.0 ml 0.05 M Tris buffer pH 9.2. The following enzyme assays were modified from Shaw and Prasad (1970): hexokinase (HK), 5.0%, buffer system five, 40 volts, 45.0 mm, stain—40.0 mg glucose, 7.0 mg $NADP^+$, 5.0 mg MTT, 20.0 units glucose-6-phosphate dehydrogenase, 1.5 mg PMS, 10.0 mg $MgCl_2$, in 25.0 ml of 0.05 M Tris buffer pH 8.0; phosphoglucomutase (PGM), 5.0%, buffer system five, 40 volts, 45.0 mm, stain—200.0 mg glucose-1-phosphate, 5.0 mg $NADP^+$, 5.0 mg MTT, 20.0 units glucose-6-phosphate dehydrogenase, 1.5 mg PMS, 60.0 mg $MgCl_2$, in 25.0 ml 0.05 M Tris buffer pH 8.0;

phosphoglucoisomerase (PGI), 5.0%, buffer system one, 70 volts, 33.0 mm, stain—40.0 mg fructose-6-phosphate, 2.5 mg $NADP^+$, 2.5 mg MTT, 20.0 units glucose-6-phosphate dehydrogenase, 0.5 mg PMS, 20.0 mg $MgCl_2$, in 25.0 ml 0.05 M Tris buffer pH 8.0; and peptidase (PEP), 6.5%, buffer system four, 40 volts, 33.0 mm, stain—5.0 mg 0-dianisidine, 5.0 mg L-amino acid oxidase, 5.0 mg peroxidase, 0.5 ml $MnCl_2$, 10.0 mg substrate (L-glycylleucine or L-leucylglycine), in 25.0 ml 0.1 M phosphate buffer pH 7.5. The following enzyme assay was modified from Hubby and Lewontin (1966): esterase (EST), 6.5%, buffer system one, 70 volts, 33.0 mm, stain—15.0 mg Fast Garnet GBC Salt, 0.5 ml substrate (1.0 g α-napthyl acetate in 50.0 ml deionized water and 50.0 ml acetone), in 25.0 ml 0.1 M phosphate buffer pH 6.5. The following assay was modified from DeLorenzo and Ruddle (1970): glutamate oxaloacetate transaminase (GOT), 5.0%, buffer system five, 40 volts, 45.0 mm, stain—100.0 mg L-aspartic acid, 50.0 mg α-ketoglutaric acid, 0.5 mg pyridoxal phosphate, 75.0 mg Fast Blue BB, 500.0 mg polyvinylpyrrolidine, in 25.0 ml 0.2 M Tris buffer pH 8.0. The assay for amylase (AMY) is modified from W. Anderson (University of Georgia, unpublished) and is as follows: 6.5%, buffer system two, 70 volts, 38.0 mm, stain—soak gel in starch substrate for 30 minutes (10.0 g Baker's potato starch, 4.54 g Tris, in 2000.0 ml deionized water, bring pH to 7.4 with HCl; heat solution until boiling or clear, and store at 5° C), wash in water and pour over gel 25.0 ml of stain (4.15 g potassium iodide, 6.25 g iodine, in 500.0 ml deionized water); let stand and wash in water. Gels stained for AMY could not be preserved.

Results

Genetic variation in the four Atlantic coast populations of *A. punctulata* was inferred from the results of twelve enzyme assays, which gave rise to more than sixteen zones of activity. The identification of homozygotes and heterozygotes was based on staining and grouping patterns and conformance to patterns demonstrated for other sexually-reproducing animals. Presumed loci were assigned an abbreviation for the enzyme name and a numeral, if more than one zone (locus) was present. The locus nearest the anode was labelled one, the next two, and so forth in order of decreasing mobility. Presumed alleles at a locus were labelled by assigning a value of 1.00 to the tracking standard and then computing the mobility difference of each appropriate band variant, *i.e.*, an allele which migrated 5.00 mm less than the standard was labelled 0.95. Twelve zones were classified genetically (Fig. 1).

Acid phosphatase (ACPH) was identified as two zones of activity, each exhibiting multiple banding. Within the slow zone (ACPH-2), single- and double-banded phenotypes were observed. A triallelic system at a single locus was proposed to account for the variation. The three alleles designated were ACPH-$2^{0.79}$, ACPH-$2^{0.83}$, and ACPH-$2^{0.87}$. Single-banded phenotypes were interpreted as homozygotes, and double-banded phenotypes as heterozygotes. The second, faster migrating zone of activity could not be classified genetically.

Malate dehydrogenase (MDH) activity was present at several bands and was grouped into two zones. Within the slow zone (MDH-2) single- and double-banded phenotypes were observed. This variation was interpreted as a diallelic system at a single locus, with single-banded patterns corresponding to the homozygous state, and double-banded patterns to the heterozygous state. The two alleles

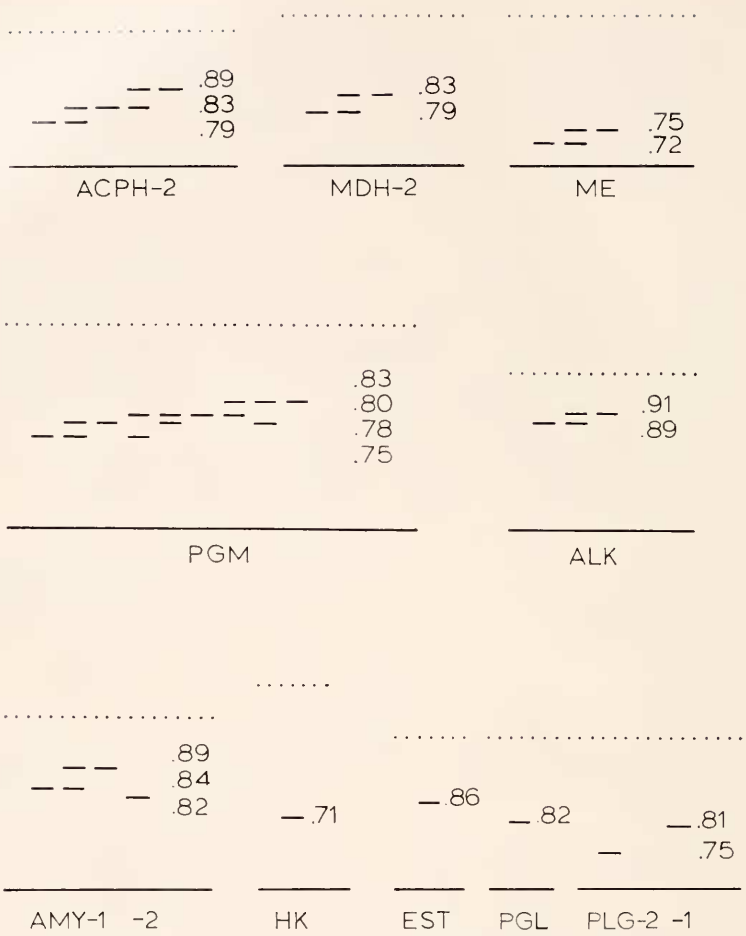

FIGURE 1. Diagram of the banding patterns for homozygotes (single-band) and heterozygotes (double-band). The origin is indicated by the solid line and migration distance of the tracking standard by the dotted line. The location of the bands and corresponding numerical value indicates the mobility relative to the standard. Designation of loci follows the text, except for PEPGL, PEPLG-1 and PEPLG-2 which are abbreviated as PGL, PLG-1, and PLG-2, respectively. The band ACPH-$2^{.80}$ is erroneously labelled and should be ACPH-$2^{.87}$.

were designated MDH-$2^{0.79}$ and MDH-$2^{0.83}$. The second faster zone could not be defined genetically.

Malic enzyme (ME) was present as a single zone of activity (ME). Single- and double-banded phenotypes were observed and were identified as homozygotes and heterozygotes respectively. Two alleles were designated, $ME^{0.72}$ and $ME^{0.75}$.

Phosphoglucomutase (PGM) was visualized as four distinct band variants within a single zone. Eight patterns were scored, consisting of either single or double bands, and these were interpreted as homozygotes and heterozygotes, respectively. The four band variants were designated allelic status as $PGM^{0.75}$, $PGM^{0.78}$, $PGM^{0.80}$, and $PGM^{0.83}$. Double-banded phenotypes expressed by individuals from

Panama City were unlike those observed in the other populations. Although the mobility of the variants corresponded to those observed for Atlantic coast specimens, the intensity of the two bands in a pair was not always alike.

Alkaline phosphatase (ALK) exhibited a complex multiple banding pattern. Only one zone of activity could be analyzed with confidence, representing a small portion of the variation detected. Within the zone of activity, single- and double-banded phenotypes were observed. These were equated with the homozygote and heterozygote classes, respectively. Two alleles were defined, $ALK^{0.89}$ and $ALK^{0.91}$.

Amylase (AMY) activity was detected as several bands which were grouped into two zones. The slow zone (AMY-2) consisted of a single heavily staining band in the majority of individuals. Fourteen urchins did not express activity in the

TABLE I

Allele frequencies and observed number of inferred genotypes for ME, MDH-2, ALK, and AMY-1. Also shown are Chi-square and probability values indicating conformance to Hardy-Weinberg equilibrium, and values of heterozygote deficiency (D_t)

ME

Population	0.72	0.75	0.72 0.72	0.72 0.75	0.75 0.75	χ^2	P	D_t
Woods Hole	0.44	0.56	1	66	9	45.20	<0.001*	0.78
Wall	0.26	0.74	0	41	38	8.81	<0.001*	0.36
Willis	0.39	0.61	4	36	16	6.19	>0.01	0.33
Beaufort	0.64	0.36	34	41	11	0.15	>0.70	0.03

MDH-2

Population	0.79	0.83	0.79 0.79	0.79 0.83	0.83 0.83	χ^2	P	D_t
Woods Hole	0.09	0.91	0	17	79	1.06	>0.30	0.06
Wall	0.26	0.74	2	36	39	3.21	>0.05	0.20
Willis	0.64	0.36	36	26	15	6.46	>0.01	−0.26
Beaufort	0.12	0.88	2	18	75	1.21	>0.20	−0.10

ALK

Population	0.89	0.91	0.89 0.89	0.89 0.91	0.91 0.91	χ^2	P	D_t
Woods Hole	1.00	0.00	95	0	0	0	>0.99	—
Wall	0.96	0.04	75	3	2	0.51	>0.30	−0.50
Willis	0.95	0.05	71	9	0	0.14	>0.70	0.18
Beaufort	0.91	0.09	65	11	1	0.10	>0.70	0.13

AMY-1

Population	0.84	0.89	0.84 0.84	0.84 0.89	0.89 0.89	χ^2	P	D_t
Woods Hole	0.58	0.42	19	75	3	33.86	<0.001*	0.60
Wall	0.57	0.43	13	64	2	31.80	<0.001*	0.64
Willis	0.60	0.40	25	37	10	0.48	>0.30	0.06
Beaufort	0.73	0.27	41	29	6	0.17	>0.50	−0.03

* Significant.

TABLE II

Allele frequencies and observed number of inferred genotypes for the ACPH-2 locus. Also shown are Chi-square and probability values indicating conformance to Hardy-Weinberg equilibrium, and values of heterozygote deficiency (D_t).

	ACPH-2									
Population	0.79	0.83	0.87	0.79 0.79	0.79 0.83	0.83 0.83	0.83 0.87	χ^2	P	D_t
Woods Hole	0	0.99	0.01	0	0	93	3	0	>0.99	—
Wall	0	1.00	0.00	0	0	80	0	0	>0.99	—
Willis	0.62	0.38	0.00	25	48	6	0	6.70	<0.01*	0.30
Beaufort	0	1.00	0.00	0	1	87	0	1	>0.99	—
Panama City	0	0.87	0.13	0	0	41	15	1.33	>0.20	0.15

* Significant.

zone. The pattern was assumed to represent monomorphism at a single locus, homozygous for one allele, which was designated AMY-$2^{0.82}$. The fast zone (AMY-1) consisted of single- and double-banded phenotypes, which as above were interpreted as representing homozygosity and heterozygosity at a single locus. Two allelic variants were designated, AMY-$1^{0.84}$ and AMY-$1^{0.89}$.

Hexokinase (HK), L-glycylleucine peptidase (PEPGL), L-leucylglycine peptidase (PEPLG), and esterase (EST) were monomorphic in all individuals. HK

TABLE III

Allele frequencies and observed number of inferred genotypes for the PGM locus. Also shown are Chi-square and probability values indicating conformance to Hardy-Weinberg equilibrium, and values of heterozygote deficiency (D_t).

	PGM				
	Population				
	Woods Hole	Wall	Willis	Beaufort	Panama City
0.75	0	0	0.01	0.07	0.02
0.78	0.10	0.05	0.11	0.22	0.13
0.80	0.10	0.13	0.33	0.25	0.21
0.83	0.80	0.82	0.55	0.46	0.64
0.75 0.75	0	0	1	0	0
0.78 0.78	5	3	5	8	3
0.80 0.80	7	11	17	16	11
0.83 0.83	50	67	29	29	35
0.75 0.78	0	0	0	7	1
0.75 0.80	0	0	0	4	2
0.78 0.80	0	0	1	1	1
0.78 0.83	3	3	1	9	8
0.80 0.83	0	0	1	1	1
χ^2	69.00	134.10	71.37	78.84	49.12
P	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
D_t	−0.85	−0.92	−0.91	−0.56	−0.61

* Significant.

TABLE IV

Chi-square and probability values for pairwise population comparisons of gene frequencies for ME, MDH-2, ALK, AMY-1, and PGM, indicating homogeneity vs. heterogeneity of allele frequencies.

Locus	Populations					
	Woods Hole-Wall	Woods Hole-Willis	Woods Hole-Beaufort	Wall-Willis	Wall-Beaufort	Willis-Beaufort
ME	12.74	1.01	11.24	5.73	47.05	15.18
	<0.001	>0.300	<0.001	>0.010	<0.001	<0.001
MDH-2	19.18	116.53	1.02	44.16	11.44	100.15
	<0.001	<0.001	>0.030	<0.001	<0.001	<0.001
ALK	9.52	11.55	15.77	0.26	1.92	0.78
	<0.010	<0.001	<0.001	>0.500	>0.100	>0.300
AMY-1	0.05	0.20	8.17	0.50	8.14	7.39
	>0.800	>0.500	<0.010	>0.300	<0.010	<0.010
PGM	2.16	21.33	35.29	24.27	52.49	11.68
	>0.500	<0.001	<0.001	<0.001	<0.001	<0.010

and PEPGL consistently stained for a single band which was interpreted as homozygosity for the alleles $HK^{0.71}$ and $PEPGL^{0.82}$, respectively. PEPLG consistently appeared as two bands in each urchin. I assumed the bands to represent two different loci, each monomorphic for a single allele. They were designated $PEPLG\text{-}2^{0.75}$ and $PEPLG\text{-}1^{0.81}$. The activity for EST was quite complex and only one zone could be interpreted. This zone was monomorphic for a single band and was assumed to represent homozygosity for a single allele $EST^{0.86}$.

Glutamate oxaloacetate transaminase (GOT) and phosphoglucoisomerase (PGI) activity could not be defined genetically.

An estimate of the amount of genetic variation present within each of the four Atlantic coast populations was calculated as the average heterozygosity over all loci per individual. This measure takes into account both the number of different alleles and the number of heterozygotes. The heterozygosity values for populations from Woods Hole, Wall, Willis Wharf, and Beaufort were 0.158 ± 0.314, 0.157 ± 0.279, 0.189 ± 0.260, and 0.123 ± 0.172, respectively.

For each polymorphic locus, gene frequencies were calculated, as well as Chi-square and probability values indicative of the conformance of the observed genotype distribution with expected values under Hardy-Weinberg equilibrium (Tables I, II, III). Also indicated are the values of heterozygote deficiency (D_t) (Koehn, Milkman, and Mitton, 1976). Significant deviations from Hardy-Weinberg equilibrium exist in each population, but not at all loci.

The results of a homogeneity Chi-square analysis of interpopulation gene frequency variation indicates significant deviation for five polymorphic loci (Table IV) (results for ACPH-2 not compared). At three of the loci (ALK, AMY-1, and PGM) a clinal shift in gene frequency is evident along the north-south transect from Woods Hole to Beaufort (Figs. 2 and 3). The gradual increase of $MDH\text{-}2^{0.79}$ which is evident in a southerly direction within the Virginian province, and of $ME^{0.72}$ between Wall and Beaufort (Fig. 2) may reflect clinal variation. A clinal trend was not observed for the ACPH-2 locus.

At the remaining loci (HK, PEPLG-1, PEPLG-2, PEPGL, EST, and AMY-

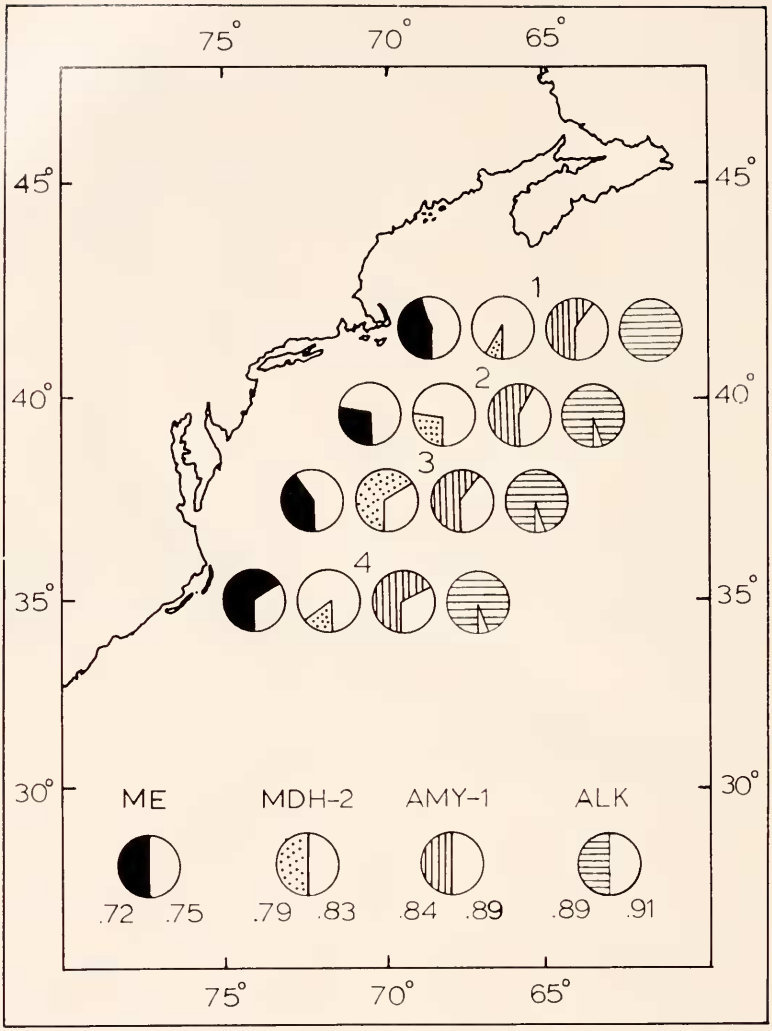

FIGURE 2. Geographic patterns of allelic variation for ME, MDH-2, AMY-1, and ALK. Designation of alleles follows the text. Numerals 1, 2, 3 and 4 correspond to Woods Hole, Wall, Willis Wharf, and Beaufort, respectively.

2), no variation within or between populations was evident. All individuals were identified as homozygotes for the same allele.

Classification of urchins according to sex was dependent upon the development of the gonads. The gonads of all specimens from Willis Wharf were reduced, and the sexes could not be distinguished. Assignment of sexual status was possible for most urchins in the other populations. This data was used to calculate gene frequencies according to sex within each Atlantic coast population for each polymorphic locus. A Chi-square test for homogeneity of allele frequencies for the sexes indicated a lack of significant variation in intersex allele frequencies.

Gene frequencies for the twelve loci (polymorphic and monomorphic) were used to determine the level of genetic identity (I′) and genetic distance (D′) (Nei, 1972) between the four Atlantic coast populations (Table V). The average genetic distance ($\bar{D}'$) for the four populations was 0.0521.

Discussion

The present study was initiated to determine the extent and pattern of genetic variation in *A. punctulata*. The data indicate significant variation in gene frequencies between the four Atlantic coast populations. Differentiation of a Gulf coast population from Atlantic populations is not striking, based upon a limited survey of four enzymes. A high level of genetic variability (0.123 ± 0.172—0.189 ± 0.260)

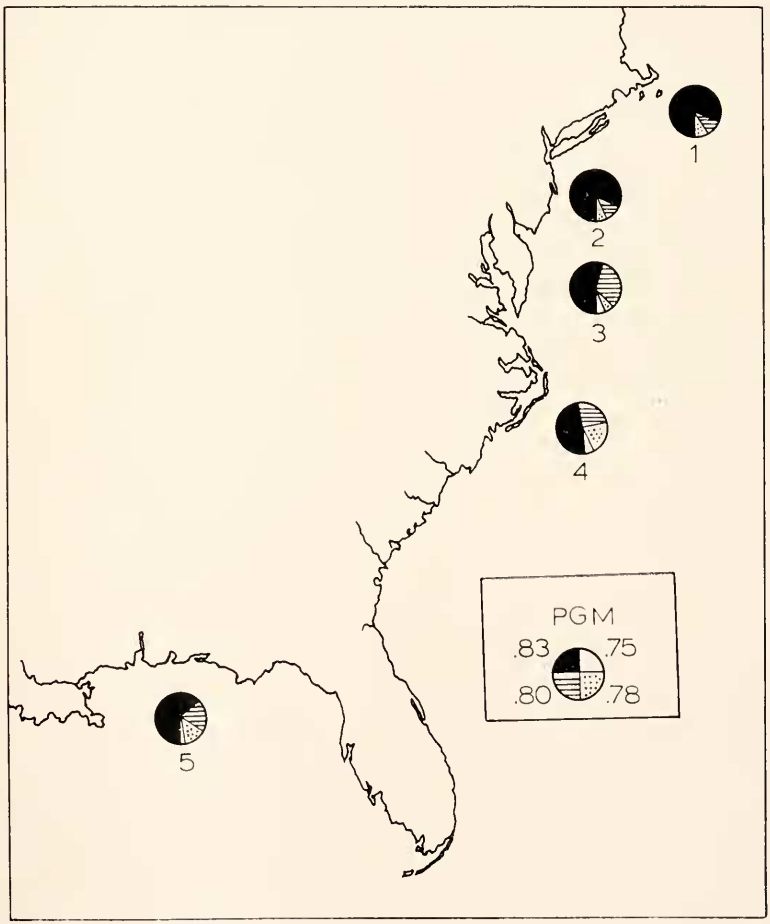

Figure 3. Geographic pattern of allelic variation for PGM. Designation of alleles follows the text, and of study sites Figure 2, with the addition of numeral 5 corresponding to Panama City.

TABLE V

The genetic identity (I') above the diagonal, and the genetic distance (D') below the diagonal are shown for each pairwise comparison of Atlantic coast populations. Also indicated is the average value of genetic distance between the four Atlantic coast populations of A. punctulata

	Woods Hole	Wall	Willis	Beaufort
Woods Hole	—	0.9929 ± 0.017	0.9171 ± 0.174	0.9812 ± 0.036
Wall	0.0071	—	0.9307 ± 0.147	0.9642 ± 0.075
Willis	0.0865	0.0718	—	0.9123 ± 0.167
Beaufort	0.0190	0.0365	0.0918	—

Average $\bar{D}' = 0.0521$.

exists within the Atlantic coast populations. The rather large standard errors for average heterozygosity indicate that the variance among loci is high, and the loci examined differ considerably in their genetic variation (see Lewontin, 1974). Heterozygosity at the ACPH-2 locus was unusually high in the population from Willis Wharf (Table II), and if this locus is excluded from the calculations of heterozygosity a value of 0.145 rather than 0.189 is obtained. All values, however, are within the range (0.024–0.202) but above the average (0.098 ± 0.022) calculated by Powell (1976) for other marine invertebrates. The higher values derived for *A. punctulata* are similar to those calculated for some plants. Selander and Kaufman (1973) report an average heterozygosity of 0.216 for four outcrossing species of Leguminosae and Compositae. A comparison of genetic variation between invertebrates and plants may be very illuminating because many sedentary marine invertebrates and plants rely on pelagic larvae or wind-borne seeds, respectively, as the primary agents of dispersal.

Several hypotheses have been proposed to account for the existence of high levels of genetic variability within and between populations (see Gooch, 1975; Levins, 1968). Powell (1971) demonstrated increased levels of genetic variability in *Drosophila* by increasing environmental heterogeneity in time and space.

The presumed adaptive nature of enzyme polymorphisms has been substantiated, although not proven, by studies which have demonstrated clinal variation in gene frequency corresponding to variation in environmental parameters (Schopf and Gooch, 1971; Murdock, Ferguson, and Seed, 1975; Johnson, 1974), selected for specific genotypes in laboratory populations exposed to different environmental regimes (Johnson, 1971; Levinton and Fundiller, 1975), and demonstrated induction of enzyme mobility variants in the same individual in response to a change in food (Oxford, 1976) and temperature (Marcus, 1977). Gene frequency clines were observed at three loci (ALK, AMY-1, and PGM) for *A. punctulata,* along the 1000 km transect between Woods Hole and Beaufort (Figs. 2 and 3). Such a trend may also typify the variation at two other loci (MDH-2 and ME). The existence of clines suggests that selection may be responsible for the maintenance of variability at the AMY-1, ALK, PGM, MDH-2, and ME loci in *A. punctulata.* The most obvious physical factor which correlates with these clinal shifts is temperature (see Bumpus, 1957). The fact that reduced clines within the full length of the transect were observed at two loci, MDH-2 and ME, indicates the interaction of several factors. It is rather doubtful that variation at different loci is the result of variation

of a single environmental factor. Most likely it is the outcome of the interaction of several environmental characteristics. Migration by larval transport may promote homogeneity of gene frequencies (Thorson, 1961; Mileikovsky, 1971; Scheltema, 1971, 1972; Gooch, Smith, and Knupp, 1972), but the fact that the magnitude and direction of allelic frequency changes are not all alike, indicates that selection against unfavorable genotypes is altering the effects of migration. The effect of migration should be the same at all loci, but the outcome of selection need not be similar.

If selection is a significant factor determining the genetic structure of *A. punctulata* populations and the species as a whole, this may explain the deviations from Hardy-Weinberg equilibrium. Wallace (1968) attributes deviation from Hardy-Weinberg equilibrium to selection, migration, mutation, and sampling error. A population formed from a mixture of individuals, derived from parental stocks which differ in terms of allele frequencies, should exhibit a net deficiency of heterozygotes in accordance with the Wahlund Effect. Many benthic invertebrate populations may originate by recruitment from several populations owing to dispersal of the planktonic larval stage in the ocean currents. Examination of the present data on *A. punctulata* which does produce a planktonic larval phase, indicates that marked heterozygote deficiencies were observed for the PGM locus only. The other loci which deviated from Hardy-Weinberg equilibrium expressed small excesses of heterozygotes. These observations tend to support the viewpoint that selection is highly important in determining the genetic structure of these populations, and for the most part over-rides the 'melting pot' effect of the larval influx from different parent populations. Heterosis may be the selective mechanism involved as indicated by the excess of heterozygotes. The variability does not appear to be due to differences in the sexes as demonstrated by the homogeneity Chi-square analysis. Size is not believed to be a factor either, because the size distribution of individuals examined was similar for all populations.

Although significant gene frequency differences were evident between the four Atlantic coast populations of *A. punctulata,* the genetic distance values between populations when all loci were considered were not high (Table V). Levels of genetic distance varied from 0.0071–0.0918 and were greatest (0.0718–0.0918) when the population from Willis Wharf was considered. Genetic distance values were less (0.0071–0.0365) for the pairwise comparisons of Woods Hole, Wall, and Beaufort populations. These values are of the same magnitude as values computed for geographic populations of other species. For example Nei (1972) calculated genetic distance values of 0.002–0.03 for geographic populations of the mouse (*Mus*); Hedgecock and Ayala (1974) calculated values of 0.005–0.053 for populations of the newt (*Taricha*); and Tracey, Nelson, Hedgecock, Schleser, and Pressick (1976) report values of 0.001–0.036 for populations of the lobster (*Homarus*). Using the data of Schopf and Gooch (1971) on the marine ectoproct (*Schizoporella errata*) I calculated values of 0.014–0.053. Genetic distance values which characterize subspecies are higher. Hedgecock and Ayala (1974) report a range of 0.109–0.253 for subspecies of *Taricha*. Genetic distance values between local populations of *Drosophila* average 0.003 ± 0.006 and between subspecies, 0.228 ± 0.026 (see Ayala, Tracey, Barr, McDonald, and Perez-Salas, 1974). The values of genetic distance for the population from Willis Wharf generally are intermediate to the

values for local populations and subspecies. The greater differentiation displayed by the population from Willis Wharf could be the result of geographic isolation and/or strong selection in a heterogeneous environment.

It appears that on the basis of allelic frequencies, genetic distance between Atlantic coast populations from Woods Hole, Wall, Willis Wharf, and Beaufort are within the range of values displayed by geographic populations of a single species. This continuity may be promoted by extensive larval transport. The success of larval transport as an agent of gene flow is dependent upon several factors, such as time of spawning, duration of life in the plankton, and current velocity and direction. Bumpus (1974) reports a general southwesterly drift for surface currents longshore from Nantucket Shoals to Cape Hatteras. In winter the velocity is maximal and on the order of 12 nautical miles/day. During the summer period, conditions such as prevailing southerly winds and reduced fresh water run-off may promote current reversals producing northerly drifts of approximately 5 nautical miles/day. Bottom drift at all times is mostly southerly and considerably slower than surface drift.

The length of larval life places one limit on how far an individual will be transported. Clearly larval life depends upon various factors such as temperature, food, and settlement responses. In the laboratory, a well fed pluteus of *A. punctulata* will metamorphose in as little as 22 days at 23°–25° C (Cameron and Hinegardner, 1974; Marcus, 1976). Larval life can be prolonged by cooler temperatures and by reduced feeding (Hinegardner, 1969, 1975). Hinegardner (1969) reports that a well-fed larva can live up to four months, but loses the capacity to metamorphose after two months. Gordon (1929) reports that larvae fed *Nitzschia closterium* began to metamorphose after 40 days, and Turner (1965) observed metamorphosis after 42 days. Thorson (1961) cites an unusually lengthy period of 97 days as the time to metamorphosis, but does not give a reference. If an average larval life of sixty days and a current velocity of 5 nautical miles per day is assumed, then a pluteus may be transported longshore during the summer a maximum distance of 558 km prior to metamorphosis. This distance represents more than half the transect between Cape Hatteras and Woods Hole. It is doubtful that surface temperatures that range from 20° C at Woods Hole to 25° C off Virginia at this time of the year would prolong larval life (Walford and Wicklund, 1968). On the other hand, juvenile and metamorphosing urchins have been collected in surface and oblique plankton tows (personal observation) in early September off Cape May and the coast of Delaware, where bottom depths are about 30 meters. Metamorphosis, therefore, need not signal the termination of transport by the ocean currents. Although the origins of the larvae collected are not known, transport of such stages would increase considerably the potential dispersal distance of the species. Because of the distance involved, migration between Willis Wharf and Woods Hole is likely to be less common than between Willis Wharf and Wall. The values of genetic distance for these populations support this hypothesis. The genetic distance between Woods Hole and Willis Wharf (0.0865) is larger than the genetic distance between Wall and Willis Wharf (0.0718). Larval exchange between Woods Hole and Wall is probably more common as seen in the small genetic distance between the two populations (0.0071).

Although Cape Hatteras is considered a barrier to the continuous distribution

of many marine organisms (Parr, 1933), genetic continuity may be promoted by the Gulf Stream and occasional spillovers of water from the Virginian province into the Carolinian province. It is reported that northeast winds in late summer and autumn promote the flow of water south of Cape Hatteras from the Virginian province (Gray and Cerame-Vivas, 1963). The southward movement of *A. punctulata* may be promoted by this system. The Gulf Stream may provide a major avenue for the transport of larvae northward to Woods Hole from the Carolinian sector. In the region of the Carolinas, *A. punctulata* is found both inshore and offshore on reefs characterized by their more tropical faunal elements (Cerame-Vivas and Gray, 1966). The presence of tropical species is attributed to the Gulf Stream which moves in a northerly direction from the Florida region. *A. punctulata* larvae may thus enter the Gulf Stream system and be transported northward. From April through September strong southerly winds cause an intermingling of coastal with Gulf Stream water, and organisms common to the Sargasso Sea are often found along the coast of Massachusetts having been blown inshore from the Gulf Stream by the winds (Sumner, Osburn, and Cole, 1911). These same forces may result in an influx of larvae from the Carolinian province. The Gulf Stream may thus be largely responsible for the great genetic similarity of the Woods Hole and Beaufort populations analyzed in this study. The greater distance observed when Beaufort urchins are compared with those from Willis Wharf supports the viewpoint recognizing Cape Hatteras as a major barrier, through which only limited dispersal can occur.

The application of this reasoning on larval transport between regions leads to the following conclusions concerning the genetic status of the populations in the Gulf of Mexico. Information on the geological history of the Gulf of Mexico and the Florida peninsula, suggests that genetic distance between the populations of the Atlantic coast and Gulf coast should be at least as great as the difference between the Beaufort and Willis Wharf populations. The Florida peninsula must act as a barrier to larval transport as does Cape Hatteras. If it is a complete obstacle to gene flow, then the populations from the Gulf of Mexico and the Atlantic have been diverging genetically ever since the emergence of the Florida peninsula, more than 30,000 years ago (see Hedgepeth, 1953). The southern part of the Florida peninsula and the Florida Keys may represent a transition zone of limited gene exchange. *A. punctulata* is rare in this region (Hedgepeth, 1953). Hydrographic data also support the premise of limited gene exchange. Parr (1935) reports that mixing between the rapidly flowing water of the Straits of Florida and the Gulf of Mexico is limited. He identifies the origin of the Florida Current from the Caribbean. This implies that any substantial exchange between the gene pools of the Gulf of Mexico and the Atlantic does not occur.

There are many species which occur both along the Atlantic and Gulf coasts, and protein variation of some of these organisms has been studied. The horseshoe crab (*Limulus polyphemus*) is polymorphic at nine of fifteen loci examined. At all but two of these loci, there are clear and consistent differences between the populations of the two coasts (Selander, Yang, Lewontin, and Johnson, 1970). Differences have also been noted for fish (Weinstein and Yerger, 1976; Johnson, 1974), and fiddler crabs (Selander, Johnson, and Avise, 1971). On the other hand, a lack of differentiation has been observed for the white shrimp (Marvin and Caillquet,

1976). Preliminary electrophoretic data of four loci of *A. punctulata* from the Gulf of Mexico does not reveal major differences between Atlantic and Gulf coast populations. Alleles or loci unique to the Gulf population were not observed. More evidence is required before any definitive answer is reached as to whether or not the results of these few loci are representative of the entire gene pool for the Gulf of Mexico.

I thank Joseph Ramus for his advice and laboratory facilities while at Yale University, and Rudolf Scheltema, Judith Grassle, and Frederick Grassle for their helpful comments and suggestions on the manuscript, which was prepared at the Woods Hole Oceanographic Institution. The work while at Yale University was supported by a USPHS grant HD 0032–13 to the author and NSF grant BMS 72-00346 to J. Ramus. Analysis of specimens from Florida was conducted at the Woods Hole Oceanographic Institution.

Summary

Genotypic variation, inferred from isozyme differences in twelve enzyme systems, was measured by means of horizontal microacrylamide gel electrophoresis in four populations of the sea urchin, *Arbacia punctulata*, from the Atlantic coast of the United States. An additional population from the Gulf of Mexico was surveyed for four enzymes. Although gene frequencies were significantly different among Atlantic populations, the overall values of genetic distance were within the range observed for geographic populations of other species. A balance between migration and selection is proposed to account for the patterns of genetic variation. Striking differences in gene frequency were not observed when Atlantic coast populations were compared with Gulf of Mexico specimens.

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